



Microplastics from biodegradable plastic bags alter soil properties and trigger stress response in sunflowers[☆]

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ABSTRACT

The widespread replacement of conventional plastics with biodegradable alternatives raises questions about their long-term effects in agricultural soils. We investigated the impact of microplastics (MPs) cryo-milled from home-compostable (PBAT_HOME) and industrial-compostable (PBAT_IND) polybutylene adipate terephthalate bags and compared it to the toxicity of MPs from conventional polyethylene (PE) bags. Sunflowers (*Helianthus annuus* L.) were grown in pots with soil spiked with 0 %, 0.05 %, 0.5 %, and 1.0 % (w/w) MPs for eight weeks under a 16/8 day-night photoperiod at room temperature (22/18 °C). Plant responses differed notably between biodegradable and conventional MPs as revealed by two-way hierarchical clustering analysis: both PBAT_HOME and PBAT_IND significantly reduced shoot and root biomass at 0.5 % and 1 %, while PE had no effect. At higher PBAT concentrations, proline levels increased, consistent with activation of drought-like stress response pathways that merit confirmation with additional molecular biomarkers. Soil water holding capacity increased at 1 % of all three MPs, indicating a lower water availability for plants in the MP-spiked soil. pH was slightly increased at 1 % PBAT_HOME and significantly at 1 % PBAT_IND soil. Despite differences in particle size, polymer composition, and starch content, no single factor explained the observed effects, suggesting a combined influence. These results highlight that biodegradable MPs can alter soil properties and trigger physiological responses in plants, emphasizing the need to assess their ecological impacts, especially in agricultural systems where soil health and resilience are critical for crop production.

1. Introduction

Plastic bags are among the most widespread forms of plastic waste globally, posing significant threats to urban landscapes, aquatic ecosystems, and other natural environments. An estimated 5 trillion plastic bags are produced annually, which corresponds to an average consumption rate of over 300 bags per person annually (Suleman et al., 2022). Alarmingly, less than 5 % of these bags are recycled globally (Knoblauch et al., 2018). These bags, primarily composed of polyethylene (PE) and derived from fossil fuels, are highly persistent in terrestrial environments (Li et al., 2022a; Yaqub et al., 2023).

Environmental exposure to sunlight, moisture, and mechanical forces leads to the gradual breakdown of plastic bags into microplastics (MPs), which have been defined as plastic fragments between 1 and 1000 µm in size according to ISO definition (ISO/DIS 24187) and less than 5000 µm in the microplastic research community (Thompson et al., 2024). To mitigate plastic pollution, biodegradable plastic bags have been promoted as eco-friendly alternatives. These bags are either produced from renewable resources (bio-based) or from fossil fuels (fossil-based) (Cucina et al., 2021; Lu et al., 2023). The most common materials used for biodegradable plastic bags include polylactic acid (PLA), polybutylene adipate terephthalate (PBAT), and starch-based polymers. PLA

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is derived from renewable resources such as corn or sugarcane, while PBAT, although primarily fossil-based, is often mixed with other biodegradable materials, such as starch, to improve its compostability and flexibility (Artru & Lecerf, 2019). While biodegradable plastic bags can degrade fully under optimal conditions, such as those found in industrial composting facilities within 90 days (Markowicz et al., 2019; Vaverková et al., 2014), studies show that their degradation is significantly slower under natural soil conditions. For example, biodegradable plastic bags have been found intact even after 27 months of burial in soil (Napper & Thompson, 2019), raising concerns about their environmental fate and effects when not disposed of properly.

Ecotoxicity studies of biodegradable plastics are polymer-specific, as MPs made of polylactic acid (PLA) have been extensively studied (Zantis et al., 2023), whereas very few data are available for other types of biodegradable MPs (Zantis et al., 2024). Recent studies reveal the complex effects of biodegradable MPs on terrestrial plants, with impacts depending on their composition, concentration, and size (Lian et al., 2022; Zantis et al., 2023). Adverse effects of both conventional and biodegradable MPs, include accumulation on roots, blocking pores, and hindering water and nutrient absorption, leading to suppressed growth, oxidative stress, disrupted photosynthesis and reduced seed germination (Baho et al., 2021; Serrano-Ruiz et al., 2023; Wang et al., 2022; Zantis et al., 2024; Zhang et al., 2023). In certain conditions, biodegradable MPs may even stimulate root growth (Sun et al., 2025). They can alter soil properties such as pH and dissolved organic carbon content, which in turn affect microbial nitrogen transformation processes and plant growth (Li et al., 2022b; Lian et al., 2022; Wang et al., 2024). Additionally, MPs can alter the soil water status, as repeatedly shown via changes in soil water holding capacity (WHC) (de Souza Machado et al., 2018; Jemec Kokalj et al., 2024; Šmídová et al., 2024). Altered WHC is particularly relevant because it can reduce water availability to plants, potentially triggering drought-like stress. Yet the direct link between MPs and plant drought responses remains poorly understood. Recent work showed that PLA MPs significantly increased proline content in maize, while PE had no such effect (Sun et al., 2023). Proline is a well-established early biomarker of drought and osmotic stress: it accumulates rapidly under water deficit, supporting osmotic adjustment, stabilizing proteins and membranes, and scavenging reactive oxygen species. Its biosynthesis is primarily regulated by abscisic acid (ABA)--dependent pathways, where ABA activates transcription factors such as DREB2A, AREB/ABF, and NAC, which in turn upregulate *P5CS1* (Δ^1 -pyrroline-5-carboxylate synthetase 1), the key enzyme driving proline accumulation (Bandurska & Stroński, 2003; Muhammad Aslam et al., 2022). Under prolonged stress, feedback mechanisms involving *ProDH1* (proline dehydrogenase 1) regulate proline turnover, integrating osmotic and oxidative stress signaling. Importantly, proline levels typically rise before higher-level indicators such as photosynthetic inhibition or growth suppression, making it one of the most sensitive markers of early drought-like stress activation. Overall, the effects of MPs are multifaceted and demand further research to mitigate potential ecosystem damage (Rillig et al., 2019).

To date, the majority of studies on the effects of MPs on terrestrial plants have investigated wheat (*Triticum aestivum* L.), corn (*Zea mays* L.) and lettuce (*Lactuca sativa* L.) (Adamczyk et al., 2024; Wang et al., 2022; Zantis et al., 2023, 2024), while other agronomically important plant species like barley (*Hordeum vulgare* L.), carrots (*Daucus carota* L.) (Zantis et al., 2024) and sunflowers (*Helianthus annuus* L.) remain underinvestigated (Okla et al., 2024). Sunflowers, the fourth most important oilseed crop globally, are notable for their drought tolerance, phytoremediation potential, and sensitivity to environmental pollutants (Hussain et al., 2018; Okla et al., 2024; Petraru et al., 2021), making them a relevant model for assessing MP-related stress response in terrestrial systems.

In this context, the main aim of this study was to assess whether MPs from biodegradable plastic bags trigger stress responses in terrestrial plant, sunflower (*Helianthus annuus* L.), and to compare these effects

with MPs from conventional fossil-based plastic bags. For this purpose, we tested MPs derived from plastic bags, which are widely used on a daily basis in the EU, namely home compostable biodegradable polybutylene adipate terephthalate (PBAT; PBAT_HOME), industrial compostable PBAT (PBAT_IND) disposable bags, and conventional polyethylene (PE) single-use bags. After eight weeks long exposure of sunflowers to MPs, we measured plant biomass, water relations, photosynthetic efficiency, and mineral composition. In addition, we quantified proline in shoots and roots as an early stress biomarker, providing new insight into potential drought-like responses. Finally, we explored whether MPs also affect selected soil properties, like pH, water holding capacity and respiration enzymes.

2. Materials and methods

2.1. Test microplastic production

One type of conventional non-degradable (PE) and two certified biodegradable (starch PBAT blend) commercially available disposable plastic bags were selected for the experiment. The PBAT plastic bags differed according to their composting certification. PBAT_HOME is certified for home composting (~20–30 °C), and PBAT_IND for industrial composting (~55–60 °C) according to EN 13432 standards (European Bioplastics Association, 2015). PE MPs were produced by cryo-milling where the milling bowl was first cooled down in liquid nitrogen for 4 min; subsequently, the milling was carried out with horizontal homogeniser (Mili Mix 20; Domel, Slovenia). The milling of the samples was performed at a horizontal frequency of 28 Hz, for 2.5 min using milling balls (diameter, 25 mm). After milling, the samples were sieved through a 250 μ m-pore-sieve. PBAT_HOME and PBAT_IND MPs were produced by cryo-milling with Ultra Centrifugal Mill ZM 300 (RETSCH, Germany) at 8000 rpm with a 1 mm stainless steel sieve. The mill and plastic material were cooled with dry ice.

Altogether, three different types of MPs were produced, hereafter named according to starting plastic bags names: PE, PBAT_HOME, PBAT_IND.

2.2. Characterisation of microplastics

For each type of MPs, more than 7500 individual particles were collected and analysed. The samples were characterised using camera (Galaxy S24 Ultra, Samsung Electronics Co., Ltd., Republic of Korea) in combination with image post-processing techniques (AxioVision, Carl Zeiss Microscopy, LLC, USA). The following parameters were calculated from the measurements: average particle size (diameter), standard deviation (SD) and shape factor (form circle). Every parameter was determined individually for each detected object (particle) in the image. The shape factor (F) was calculated as Equation (1):

$$F = \frac{4\pi A}{P^2} \quad (1)$$

where A is the particle area and P its perimeter. Values close to 1 indicate circular particles, while lower values represent increasingly irregular or elongated shapes.

The diameter is calculated by the software as the diameter of a circle with equivalent area as the analysed particle. The final value for each parameter represents the arithmetic mean of all measured values in the analysed population.

Fourier transform infrared spectroscopy (FTIR) was used for chemical characterisation of three types of produced MPs (Rathore et al., 2023). The spectra were collected with a LUMOS II FTIR Imaging Microscope (Bruker, USA) in ATR mode with an MCT detector, cooled with liquid nitrogen (resolution 4 cm^{-1} , 64 scans). The wavenumber of obtained spectra ranged from 3998 cm^{-1} –599 cm^{-1} .

To investigate the surface morphology and microstructural features

of the observed MPs samples (PE, PBAT_HOME and PBAT_IND), detailed characterisation was performed using a high-resolution field emission scanning electron microscope (FE-SEM; Apreo 2, Thermo Scientific, Thermo Fisher Scientific, USA). Prior to imaging, samples were carefully mounted on aluminum sample holders with conductive carbon tape to ensure stability. To improve surface conductivity and minimize charging effects during electron beam exposure, an approximately 10 nm thick conductive coating of a gold/palladium (Au/Pd) alloy was uniformly applied to the sample surfaces using the Q150T ES high-resolution sputter coater (Quorum, UK). SEM imaging was performed with an accelerating cathode voltage of 2 kV, ~8 mm working distance and a detector for secondary electrons.

Thermogravimetric analysis (TGA) was conducted on a Mettler Toledo TGA/DSC 1 thermogravimeter. Each sample, PE (11.08 mg), PBAT_IND (9.89 mg), and PBAT_HOME (10.21 mg) was analysed under a nitrogen atmosphere with a flow rate of 20 mL/min and a heating rate of 10 K/min from 50 to 600 °C. The analysis was then continued from 600 to 1000 °C under an oxygen atmosphere, maintaining the same flow rate and heating rate (Xiang et al., 2020).

2.3. Spiking of soil with microplastics

The following concentrations of MPs (PE, PBAT_HOME, PBAT_IND) were tested: 0.05 %, 0.5 % and 1 % (w/w dry soil weight). These concentrations were chosen to cover the range according to values already found in agricultural soil (Zhang et al., 2018) to worst-case scenario concentrations (Büks & Kaupenjohann, 2020) and are also in the range of those previously used in our studies (Jemec Kokalj et al., 2022, 2024; Selonen et al., 2020, 2021). It is predicted that the concentration of MPs in agricultural soil could reach 0.67 % (w/w) within the next century (Meizoso-Regueira et al., 2024). MPs were thoroughly mixed into dry standard agricultural Lufa 2.2 loamy sand soil (Lufa Speyer, Germany) (properties in Table S1) using a metal spoon. Metal equipment was used to handle the soil and MPs to avoid contamination. This procedure was previously used in our experiments (Jemec Kokalj et al., 2024). Soil mixed with MPs was distributed in the pots on the same day as prepared. In the control group, the sunflowers were exposed to untreated Lufa 2.2 soil and are referred as 0 % throughout the text.

2.4. Plant species and cultivation

Sunflower (*Helianthus annuus* L.) seeds were obtained from Vitakraft pet care GmbH & Co. KG, Bremen and were first planted into the vermiculite to obtain seedlings. Ten days after germination, the equally sized seedlings were removed from the substrate, the roots were washed with water to remove the substrate and planted individually into experimental pots (7x7x7 cm) containing 270 g of dry Lufa 2.2 soil, pre-mixed with different types and concentrations of MPs.

2.5. Plant pot experiment

Ten replicates for each MP treatment and the control were prepared. One sunflower seedling was planted in each pot containing 270 g of dry soil with different concentrations and types of MPs. Initially, each pot was watered from above with 100 mL of water, and after gravitational water drainage, an additional 20 mL was added to the tray beneath the pot to avoid drying out. The exposure took place for eight weeks under controlled conditions in a growth chamber (constant light with photon flux 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 16/8 day-night photoperiod, temperature 22/18 ± 1 °C and 60 % relative humidity). Watering was done twice per week on the top of the soil (20 mL), and in the tray beneath the pot.

During the experiment, the height of the shoots was measured once per week on the same day, from the point where the shoot emerged from the soil to the highest leaf in its natural position. We collected the soil from the whole pot for further analysis because it was not possible to separate rhizosphere soil from the rest (the roots completely overgrew).

2.6. Measuring soil properties after spiking with microplastics

Measurements of water holding capacity (WHC) and pH were conducted for the control soil as well as for all types of MPs and their respective concentrations. WHC was measured only once, at the start of the experiment on freshly prepared soil, because later measurement was impossible due to extensive root growth, which could not be separated from the soil. WHC was measured according to Annex in the ISO 11268-2 (2023). The tube, with a diameter of 3 cm, was plugged at the bottom with filter paper. Approximately 5 cm of soil was put inside and placed in a water bath for 3 h. The sample was further placed for a period of 2 h on very wet, finely ground quartz sand for draining. Wet soil was taken out of the tubes and weighed. After drying at 105 °C until a constant mass, the dry mass was recorded. WHC was calculated as the difference between the mass of the water-saturated substrate and dry mass of the substrate compared to dry mass of the substrate according to Equation (2):

$$\text{WHC} = \frac{m_s - m_d}{m_d} \cdot 100 \quad (2)$$

where, WHC is water holding capacity in %, m_s is the mass of the water-saturated soil and m_d is the dry mass of the soil. Measurements were done in triplicates.

Soil pH was measured at the beginning and at the end of the sunflower experiment (after eight weeks). Soil was collected from different pots of the same treatment and combined together. Measurements were made in technical triplicates. pH was measured according to OECD TG 222 (2016). 25 mL of 0.01 M calcium chloride (CaCl_2) solution was added to 5 g of soil and left to settle for 2 h. The pH of the liquid phase was then measured using a pH meter (SevenCompact, Mettler Toledo).

The activities of soil enzymes (dehydrogenases, ureases) were also measured in soil with MPs. Dehydrogenase (DH) activity was determined in accordance with the standard EN ISO 23753-1:2019. Briefly, 5 g of moist soil sample was weighed into a 50 mL tube. Then 4 mL of tris (hydroxymethyl)aminomethane (Tris) buffer solution (100 mM, pH 7.6) and 1 mL of 2,3,5-triphenyltetrazolium chloride (TTC) substrate solution (300 mM) were added into the sample tube. For control tubes, only 4 mL of Tris buffer solution was added. The tubes were then shaken manually for a few seconds, sealed with caps and incubated at 25 °C for 6 h. To extract formed triphenylformazan (TPF), 25 mL of ethanol (96 %) was added, and tubes were then incubated for 1 h in the dark on an orbital shaker (Tehtnica RVI-403, Slovenia) (250 min^{-1}) at room temperature. After the incubation, 1 mL of TTC substrate solution was added to the control tubes. All tubes were then centrifuged for 5 min at 2000 g (Domet, Centric 350, Slovenia). The absorbance of supernatant was then measured spectrophotometrically (Varian, Carry 50 Probe, UV-Visible Spectrophotometer) at 485 nm. DH activity was calculated according to equation (3):

$$A = \frac{(C_s - C_b) \cdot V \cdot 100}{m \cdot \text{DM} \cdot \text{RT}} \quad (3)$$

where A is the enzymatic activity in mU/g of each dry sample replicate, C_s is the concentration of TPF formed in sample replicates in nmol/mL, C_b is the concentration of TPF formed in the control tube in nmol/mL, and V is reaction volume in mL, RT is reaction time in min, m is the soil mass per tube in g, DM is the sample dry matter content in accordance with ISO 11465 in %.

Urease (UR) activity was determined by measuring ammonia formed from the hydrolysis of urea. Briefly, 5 g of moist soil sample was weighed into 100 mL Erlenmeyer flask. Then 2.5 mL of urea solution (0.08 M) was added to the flask. For the control sample, 2.5 mL of deionized water was added instead. Erlenmeyer flasks were sealed and incubated at 37 °C for 2 h. After incubation, hydrolysis was stopped by adding 50 mL of 1.0 M KCl and 0.01 M HCl mixture solution. 2.5 mL of urea solution was added to the control sample just before adding KCl and HCl mixture

solution. Flasks were placed on a magnetic stirrer for 30 min. Samples were then filtered in a dark room, and 1 mL of filtrate was taken and diluted to 10 mL with deionized water. The concentration of formed ammonia was measured spectrophotometrically with UV-Vis microplate reader Biotek Synergy/LX multi-mode reader at 655 nm in accordance with the standard ISO 7150-1:1984. The concentration of NH_4^+ was calculated from the calibration curve. UR activity was expressed as μg of hydrolysed nitrogen per g of dry sample.

2.7. The analysis of physiological and biochemical parameters of sunflowers

After seven weeks, the transpiration rate (in $\text{mmol m}^{-2} \text{s}^{-1}$) was measured on all plants (ten replicates per treatment) on the first fully developed leaf. A leaf porometer (Team Medical & scientific Sdn. Bhd.) was used for the measurements. The porometer was placed on the surface of the leaf, taking care to avoid placing it on the midrib.

The efficiency of photosystem II was measured after seven weeks of exposure on the first fully developed leaf (ten replicates for each treatment). The potential efficiency of photosystem II (F_v/F_m) was measured on leaves that were kept in darkness for 15 min, and the actual efficiency of photosystem II (Y) was measured on the leaves that were exposed to light. The measurements were performed with a PAM-2500 fluorometer (Heinz Walz GmbH).

After eight weeks of growth, the plants were removed from the soil, and the roots were separated from the shoots. The root and shoot fresh biomass were first measured. Then, the roots and shoots were frozen in liquid nitrogen and lyophilised (CoosSafe 110-4, ScanVac, Labogene, Denmark) for 72 h, and the dry biomass was measured. The water content of plants was calculated by comparing the difference between the fresh and dry shoot and root biomass following Equation (4) (Turner, 1981):

$$WC = \frac{m_F - m_D}{m_F} \cdot 100 \quad (4)$$

where WC is water content in %, m_D is dry weight (of shoots and roots), and m_F is fresh weight (of shoots and roots).

The lyophilised roots and shoots were pooled in groups of three, ground in a mortar with liquid nitrogen, and pressed into pellets for the measurements of mineral composition. Three pellets per treatment were pressed from lyophilised material using a pellet die and manual hydraulic press (Specac, UK) and measured by a portable XRF X-ray fluorescence spectrometer, Peduzo P01 (Jozef Stefan Institute, Slovenia) with Rh X-ray tube and SDD (Amptek, USA) detector. The tube energy was set to 35 keV. The beam was collimated to 5 mm, and the energy resolution was 140 eV at 5.6 keV. The quantitative analysis was performed by the Quantitative X-ray analysis system software package developed in LabView (Kump, 2024). The results were validated using standard reference material (tomato leaves NIST SRM 1573a).

Proline content was measured in lyophilised roots and shoots according to the method of Bates et al. (1973). Approximately 0.1 g of the dry sample was weighted and 2 mL of a 3 % sulphosalicylic acid solution was added. The mixture was centrifuged for about 10 min 1 mL of the supernatant was pipetted into a test tube, and glacial acetic acid and an acid solution of ninhydrin (1:1:1) was added and incubated for 1 h at 85 °C. The reaction was stopped by placing the reaction mixture in an ice bath. The absorbance of the organic phase was measured at 520 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800 UV-Vis Spectrophotometer, Japan). The proline concentration was determined according to a standard L-proline standard curve with concentrations of 1–20 $\mu\text{g/mL}$. The results were expressed as μg of proline per g of dry weight of the sample.

2.8. Data analysis

The data were analysed and visualised using the GraphPad Prism 10 software (GraphPad Software, LCC, Boston, MA, USA). All data were first tested using the Shapiro-Wilk test to determine whether they met the assumptions for normality and homogeneity of variance. Depending on the outcome of these later tests we used Kruskal-Wallis ANOVA followed by Dunn's test ($p \leq 0.05$) or One-way ANOVA followed by Tukey's test ($p \leq 0.05$). Only data with statistically significant differences between the two treatments are shown, the rest are presented in the Supplementary material (Table S1, Table S2, Table S3, Table S4).

Two-way hierarchical clustering based on Euclidean distance with a heat chart was performed on z-normalized data (value of the parameter – mean of the parameter/SD of the parameter, $(x - \mu) \sigma^{-1}$) in "R" (R_x64 3.6.0), using 'gplots' including all measured parameters and all treatments (Ultsch & Löttsch, 2022).

3. Results

3.1. Microplastic characteristics

The results show that the average particle size varies between the three MP samples analysed. The PE, PBAT_HOME, and PBAT_IND had average particle sizes $629 \pm 537 \mu\text{m}$, $873 \pm 613 \mu\text{m}$ and $991 \pm 636 \mu\text{m}$ (mean $\pm$ SD), respectively, indicating wide particle size distribution (Fig. 1A).

The diameters of the three MPs were statistically significantly different ($p \leq 0.001$). All particles were irregularly shaped, in fact, the shape factors of all three samples were around 0.7, indicating that the particles are similarly irregular and slightly elongated in shape, with no significant differences between the samples. This observation is consistent with the SEM images (Fig. 1C), on which all particles appeared non-spherical. In particular, the PE particles were slightly smaller, wavy and had a smoother surface compared to the PBAT-based particles.

Fig. 1B shows the FTIR spectra of the prepared MPs. Based on the spectra library of the software OPUS (Bruker, USA) it was confirmed that prepared PE MPs were made of high-density polyethylene (HD-PE) with characteristics peaks at 2915 cm^{-1} (asymmetric stretching), 2847 cm^{-1} ($-\text{CH}_2$ symmetric stretching), 1462 cm^{-1} ($-\text{CH}=\text{CH}-$ stretching) and 719 cm^{-1} ($-\text{CH}_2$ rocking) (Campanale et al., 2023). Spectra of PBAT_HOME and PBAT_IND are similar to each other and to spectra of polybutylene adipate terephthalate (PBAT) with peaks around 3000 cm^{-1} (C-H stretching), 1710 cm^{-1} (C=O), 1267 cm^{-1} (C-O) and 720 cm^{-1} ($-\text{CH}_2-$) (Al-Itty et al., 2012). The spectra of PE, PBAT_HOME and PBAT_IND are also similar to the spectra of PE and PBAT MPs we used in our previous study (Putar et al., 2025).

TGA curves for PE, PBAT_IND, and PBAT_HOME, along with the corresponding first derivatives are shown in Fig. S1. The TGA curve for PE indicates an onset temperature of 435 °C, a 50 % decomposition temperature at 480 °C, and an inflection point (T_p) at 484 °C. The TGA curves for PBAT_IND, and PBAT_HOME show two major mass-loss steps coinciding with the temperature ranges for starch and PBAT thermal degradation: the T_p values for starch and PBAT are 328 °C and 406 °C in PBAT_IND, and 317 °C and 405 °C in PBAT_HOME. The T_p of starch typically ranges from 300 to 350 °C, depending on botanical origin (e.g., corn, rice, potato, pea, maize, etc.) (Liu et al., 2013). The mass loss between 200 and 350 °C corresponds to the decomposition of starch content (Karimi & Biria, 2019; Phillips & Blazey, 1991). Using this range, the starch content in PBAT_IND and PBAT_HOME is 23 % and 24 %, respectively. First derivative curves indicate good separation between the decomposition of both components and indicate maximum decomposition temperatures of starch and PBAT.

3.2. The effect of microplastics on soil properties

WHC was measured in Lufa 2.2 soil for all treatments before the

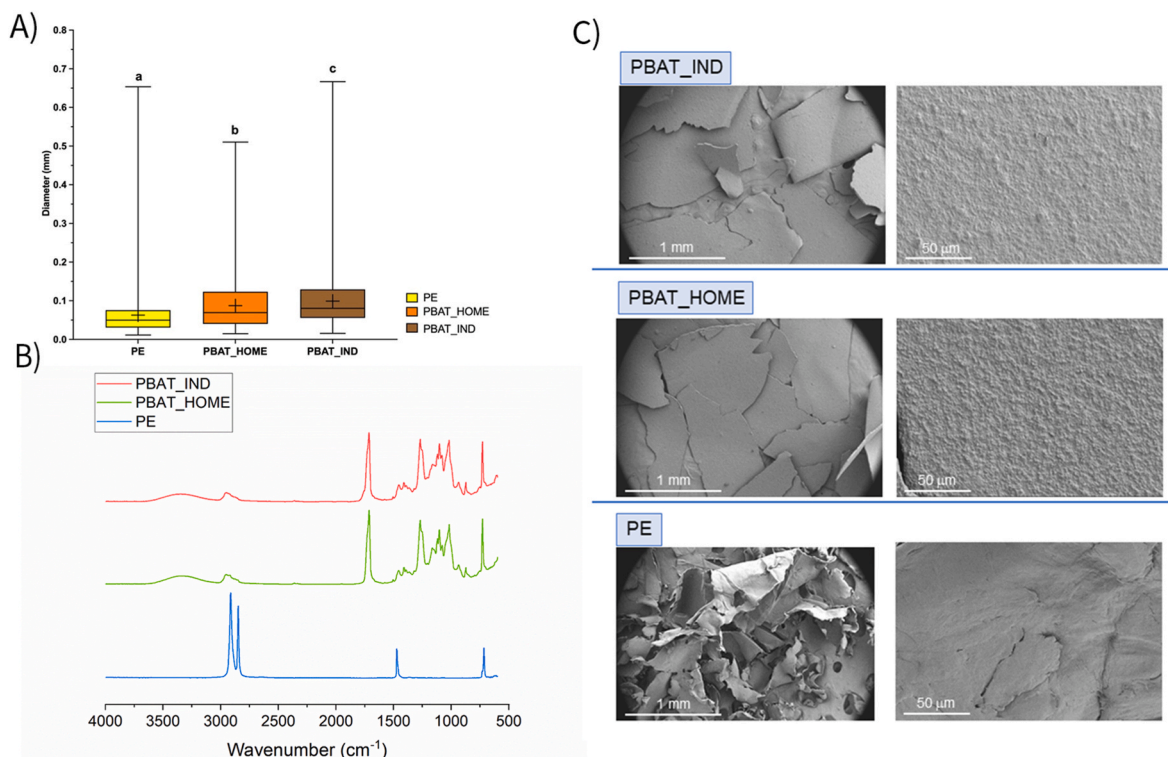


Fig. 1. Characterisation of microplastics used in this study. (A) Particle diameter distributions for polyethylene (PE), home-compostable polybutylene adipate terephthalate (PBAT_HOME), and industrial-compostable PBAT (PBAT_IND), shown as box-and-whisker plots (plus = mean; line = median; whiskers = minimum–maximum). Different letters denote significant differences among polymers ($p < 0.05$). (B) FTIR spectra confirming chemical identity of the polymers. (C) Scanning electron micrographs of PBAT_IND, PBAT_HOME and PE samples (left) and corresponding surface morphologies at higher magnification (right); scale bars as indicated.

experiment. A significant increase of WHC was found at 1 % concentration of PE, PBAT_HOME and PBAT_IND compared with the control (Fig. 2A). The pH at the beginning of the experiment did not differ between the control and different concentrations of MPs (not shown). However, at the end of the experiment a significant difference in pH relative to the control (pH = 7.2) was observed at 1 % PBAT_IND (pH = 7.4) (Fig. 2B). Nevertheless, a trend of increased pH can also be observed for PBAT_HOME (pH = 7.3).

3.3. The effect of MPs on soil enzymes

The addition of MPs to the soil significantly ($p \leq 0.05$) affected DH

and UR activity in a type- and complex non-monotonic manner (Fig. 3). Namely, at 0.05 % and 0.5 % PE, DH activity increased by 53.6 % and 24.1 % compared to the control, whereas at 1 % PE, DH activity decreased by 58.8 %. For 0.05 % PBAT_HOME, DH activity increased by 47.5 %, but at 0.5 %, it decreased by 35.7 %. In the case of PBAT_IND, DH activity decreased significantly by 5.6 %, 72.9 % and 10.2 % across all three concentrations (0.05 %, 0.5 % and 1 %), respectively (Fig. 3A).

In contrast, UR activity increased significantly, by 221.9 % and 306.4 %, at 0.05 % and 1 % PBAT_IND concentrations compared to the control, respectively. However, no significant differences were observed for PE and PBAT_HOME (Fig. 3B).

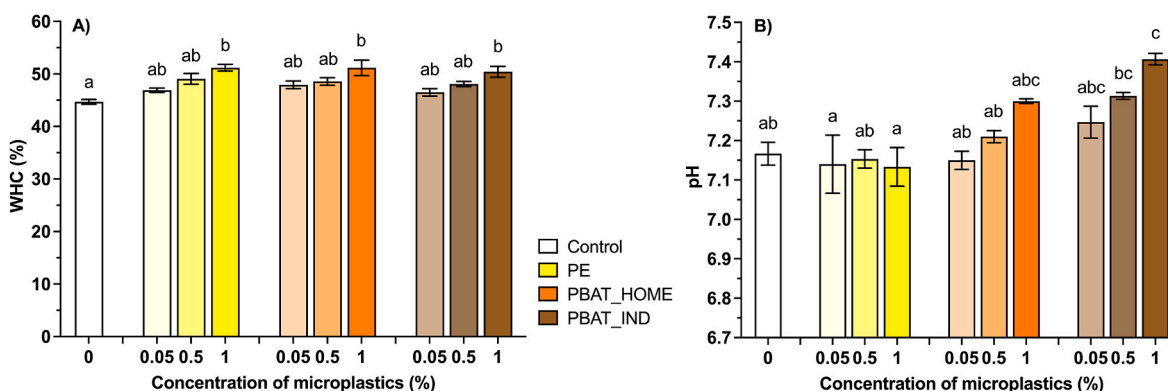


Fig. 2. Water holding capacity (WHC; A) and pH (B) of Lufa 2.2 soil with different concentrations of microplastics (MPs; 0 %, 0.05 %, 0.5 % and 1 % w/w; MPs weight per dry soil weight). WHC and pH were measured in soil with polyethylene (PE) and polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) MPs. WHC was measured on freshly spiked soil with MPs, while pH was measured at the end of the experiment with sunflowers (after 8 weeks of exposure). Columns represent mean $\pm$ standard error. Different letters denote statistically significant differences among treatments ($p \leq 0.05$).

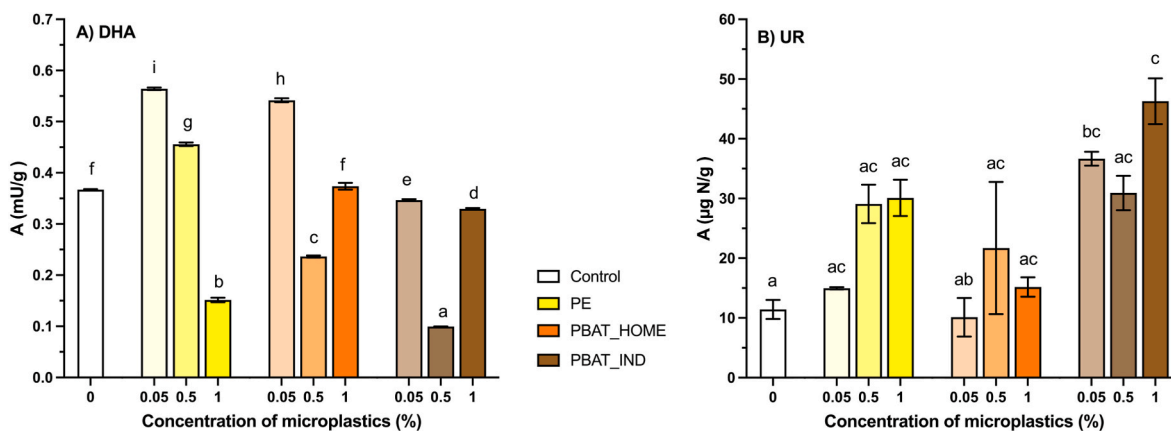


Fig. 3. Dehydrogenase (DH; A) and urease (UR; B) activities in soils spiked with polyethylene (PE) or polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) MPs at different concentrations (0 %, 0.05 %, 0.5 % and 1 % w/w; MPs weight per dry soil weight). Columns represent mean $\pm$ standard error (N = 3 for DH, and N = 6 for UR). Different letters denote statistically significant differences among treatments ($p \leq 0.05$).

3.4. Plant physiological responses

The plant growth rate, water content, transpiration rate, potential (F_v/F_m) and actual photosynthetic efficiency (yield; Y) showed no statistically significant differences between the treatments and the control group at any concentration or type of MP (Table S2). The values were comparable between treatments, indicating that MPs did not significantly affect these parameters at the studied concentrations.

The root and shoot fresh and dry weights were measured at the end of the experiment. Results showed that, except for the shoot dry weight, there were no statistically significant differences between the control and all the treatments. In the case of shoot dry weight (Fig. 4B), there was a statistically significant decrease (23.5 %) at both 0.5 % and 1 % PBAT_IND MP relative to the control. However, when comparing MP treatments, we observed that PE MP, especially at the highest concentration, induces a different response than PBAT_HOME and PBAT_IND at concentrations of 0.5 % and 1 %. Namely, at 1 % PE, the fresh and dry biomass of shoots and roots was higher compared to PBAT_HOME and PBAT_IND (Fig. 4). Therefore, although no significant differences were found between the treatments and the control, a trend was observed in which PE at the highest concentration promoted root and shoot growth, while both PBAT materials decreased growth.

3.5. Effects of microplastics on mineral nutrient uptake

The concentrations of chlorine (Cl), iron (Fe), calcium (Ca), phosphorus (P), and sulfur (S), in both shoots and roots did not show statistically significant differences in comparison to the control nor across different MP treatments (Table S3 and Table S4), indicating that the presence of MPs in the soil did not substantially affect the uptake of these elements. Similarly, no significant changes were observed in shoot potassium (K) or root zinc (Zn) concentrations relative to the control, suggesting that these elements were also largely unaffected by MPs exposure under the tested conditions. In contrast, statistically significant differences were observed between MPs treatments in shoot K, root Zn and root manganese (Mn) concentrations. A significant difference in shoot K concentration was found between plants exposed to PBAT_IND at 0.5 % and PE at 1 %, indicating that the type of MPs may differentially affect K availability or uptake (Fig. 5A). Similarly, although root Zn concentration differed significantly among treatments (Fig. 5C), no consistent pattern emerged in relation to MPs type or concentration, and none of the treatments showed a significant difference compared to the control. The only element showing a difference with the control was the Mn concentration in roots which increased significantly in PBAT_HOME MPs at 0.5 % and 1 %, by 95 % and 72 %, respectively (Fig. 5B). These treatments were also significantly different from 0.05 % to 0.5 % of PE suggesting that biodegradable MPs, particularly PBAT_HOME, may enhance Mn availability at certain concentrations, while conventional

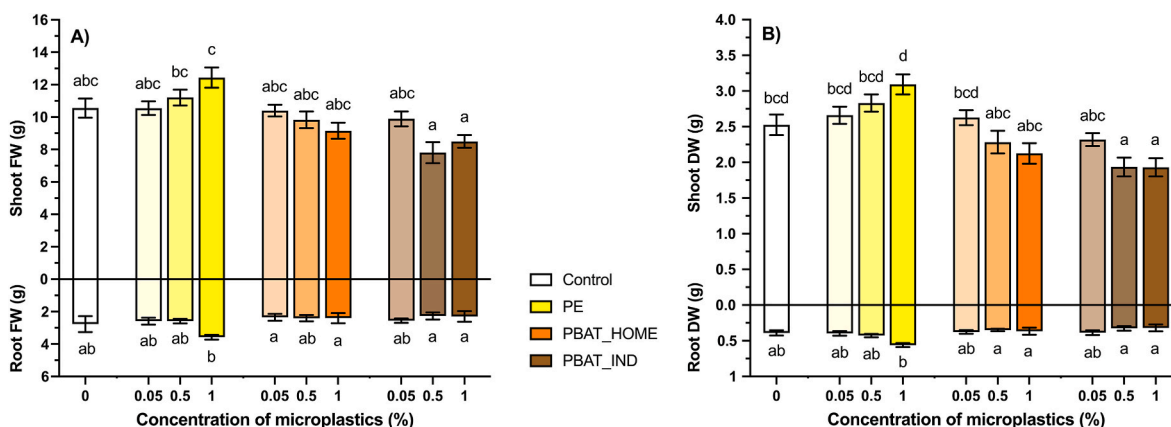


Fig. 4. Shoot and root fresh (A) and dry (B) weight of sunflower seedlings. Sunflowers were grown in soil with polyethylene (PE) or polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) at different MP concentrations (0 %, 0.05 %, 0.5 % or 1 % MPs weight per dry soil weight). Measurements were taken after eight weeks of plant growth. Columns represent mean $\pm$ standard error (n = 9–10). Different letters denote statistically significant differences among treatments ($p \leq 0.05$).

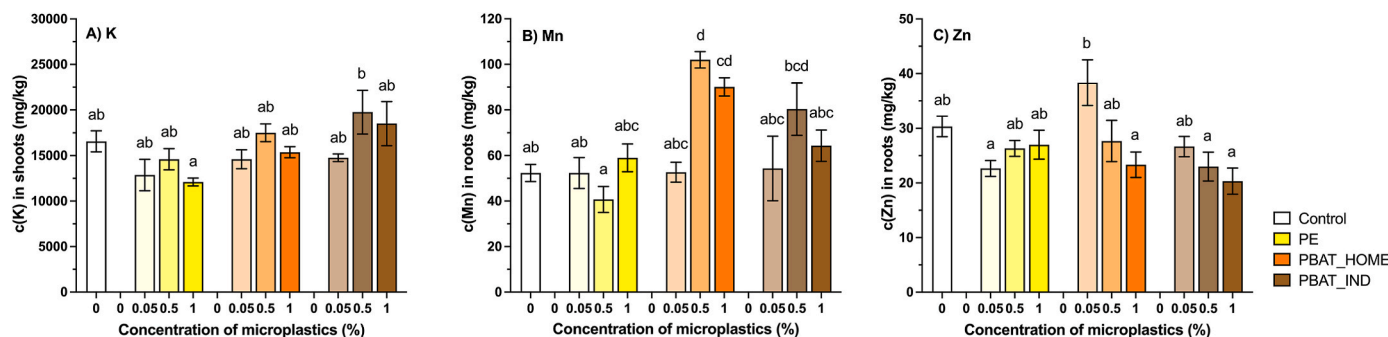


Fig. 5. Concentration of potassium (K) in shoots (A) and manganese (Mn; B) and zinc (Zn; C) in sunflower roots. Sunflowers were grown in soil with polyethylene (PE) or polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) at different concentrations (0 %, 0.05 %, 0.5 % or 1 % w/w; MPs weight per dry soil weight). Measurements were taken after eight weeks of plant growth. Columns represent mean $\pm$ standard error (N = 3). Different letters denote statistically significant differences among treatments.

MPs may reduce it.

3.6. Proline accumulation

Exposure to MPs significantly increased proline accumulation in both roots and shoots of sunflower plants (Fig. 6). In the roots (Fig. 6A), the highest proline content was observed in plants treated with 1 % PBAT_IND, showing a significant increase compared to the control and all the other treatments (except for 0.5 % PBAT_IND). In the shoots the most pronounced increase was observed in plants treated with 0.5 % and 1 % PBAT_HOME and PBAT_IND, both of which had significantly higher (up to 103 %) proline levels compared to the control (Fig. 6B).

4. Discussion

This study aimed to assess how MPs from conventional and biodegradable plastic bags influence soil properties and plant health, focusing on sunflower stress responses. We compared MPs from two types of biodegradable bags (PBAT_HOME and PBAT_IND) and from one type of conventional (PE) plastic bag.

4.1. Effects of microplastics on plant physiology

A set of parameters indicating plant performance was measured. While actual and potential efficiency of photosystem II, growth rate, water content and transpiration rate were not altered by MPs exposure, we observed marked effects on root and shoot biomass. We showed that different MPs impact sunflower biomass in distinct ways (Fig. 7). While

PE MPs at higher concentrations (1 % w/w) appeared to promote both shoot and root biomass, PBAT-based MPs, particularly PBAT_IND at 0.5 % and 1 %, had a negative effect on plant growth. The latter aligns with previous research indicating that biodegradable plastics can negatively affect plant growth (Lincmaierová et al., 2023; Zhang et al., 2022). The suppression of root biomass in PBAT-treated plants at 0.5 % and 1 % suggests that these MPs may hinder root elongation and branching (Lozano et al., 2021; Yuan et al., 2024). In contrast, the increase in both fresh and dry root weights for PE at 1 % concentration suggests that PE MPs may enhance root growth, potentially due to improved soil aeration and water retention (Lozano & Rillig, 2022). These findings highlight that the effects of MPs on plant growth are not uniform.

4.2. Effects of microplastics on mineral nutrient uptake

Nutrient availability and uptake by plants are crucial for growth and overall physiological health, but to date, there are very few studies that have analysed the effects of MPs on the mineral composition of plants (Ruan et al., 2024). Our results demonstrate that MPs contamination in soil can influence nutrient levels in sunflower shoots and roots, with distinct effects observed for different types and concentrations of MPs. While the level of nutrients, with the exception of Mn, was not different compared to control, the concentration of K in shoots, Mn in roots and Zn in roots varied significantly among PE and PBAT treatments. This study highlights the need for further research on how MPs affect nutrient bioavailability and element distribution in plant systems.

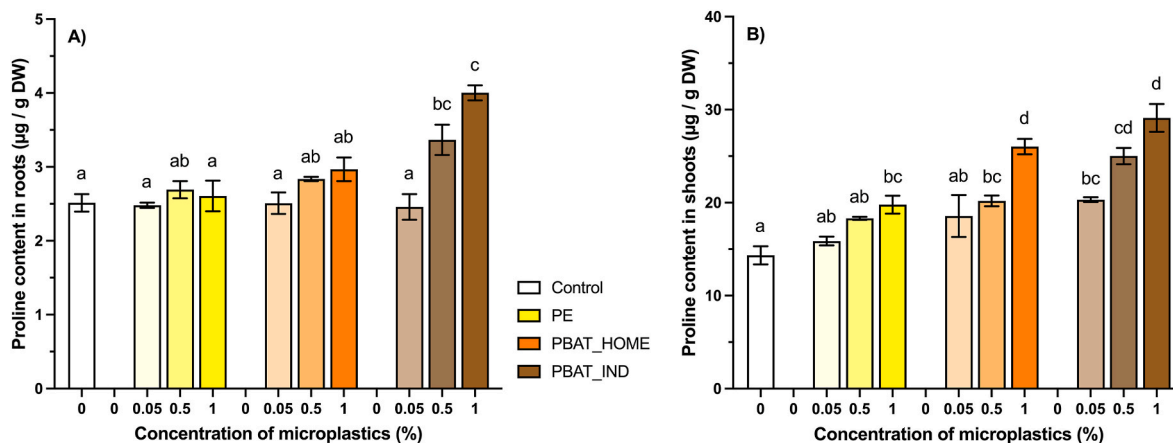


Fig. 6. Proline content in roots (A) and shoots (B). Sunflowers were grown in soil with polyethylene (PE) or polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) at different concentrations (0 %, 0.05 %, 0.5 % or 1 % w/w; MPs weight per dry soil weight). Measurements were taken after eight weeks of plant growth. Columns represent mean $\pm$ standard error (N = 3). Different letters denote statistically significant differences among treatments.

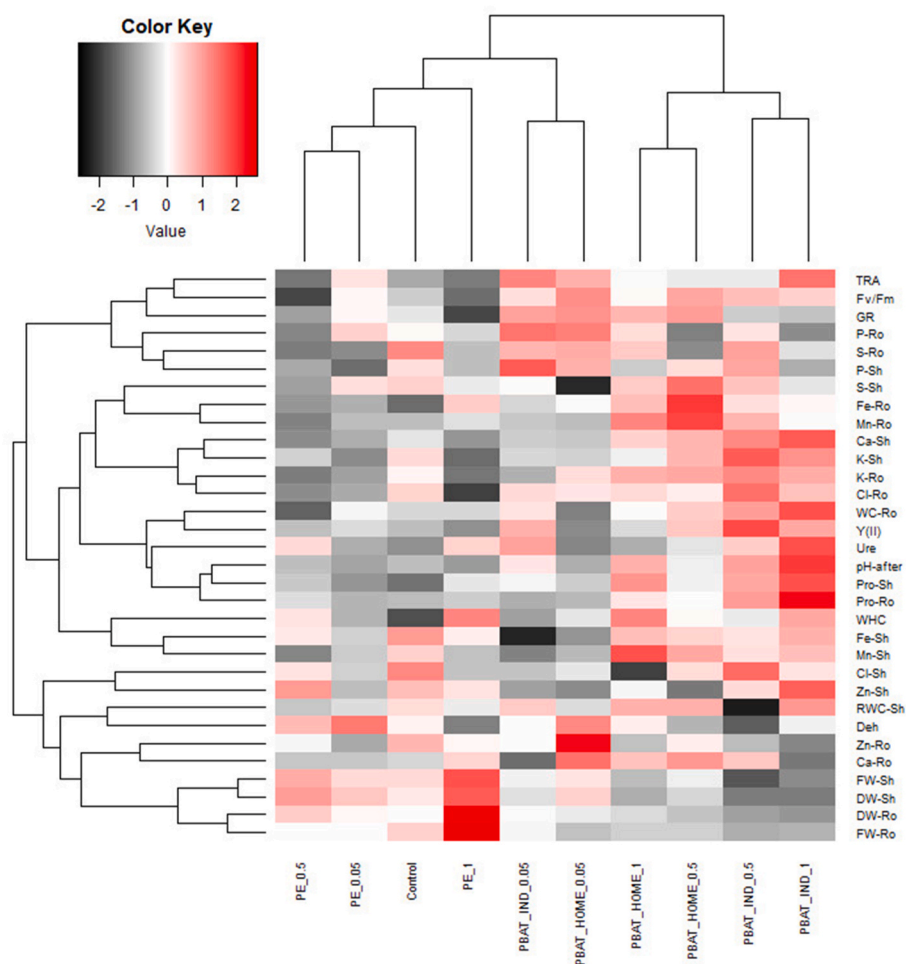


Fig. 7. Heatmap with two-way hierarchical clustering performed on z-normalized physiological, biochemical and growth parameters of sunflowers treated with different types and concentrations of microplastics (MPs) and soil WHC, enzyme activity and pH. The color gradients on the heatmap show the deviations of each parameter from the mean z-value, with red indicating higher z-values values, black lower z-values values and white z-values values close to 0. The different MP treatments, including conventional polyethylene (PE) and polybutylene adipate terephthalate (PBAT_HOME and PBAT_IND) at different concentrations (0 %, 0.05 %, 0.5 % or 1 % w/w), are ranked according to the similarity of their effects on the selected parameters (Ro – roots; Sh – shoots; FW – fresh weight; DW – dry weight; GR – growth rate; Y – actual efficiency of photosystem II; Fv/Fm – potential efficiency of photosystem II; TRA – transpiration rate; Pro – proline concentration; Cl – chlorine concentration; Zn – zinc concentration; S – sulfur concentration; Fe – iron concentration; Mn – manganese concentration; K – potassium concentration; Ca – calcium concentration; P – phosphorus concentration; Ure – urease activity; Deh – dehydrogenase activity). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.3. Proline accumulation

The most notable finding of this study was the strong accumulation of proline in both roots and shoots exposed to higher concentrations of biodegradable PBAT MPs. Elevated proline, a well-established drought-like stress biomarker, has been associated with osmotic and oxidative stress in sunflower (Canavar et al., 2014) and other plant species. Similar responses were reported in maize exposed to biodegradable MPs, where polylactic acid (PLA) MPs significantly increased proline levels, while PE had no such effect (Sun et al., 2023). Although photosynthetic and plant water-related parameters remained unchanged in our study, the concurrent increase in proline and decrease in biomass under PBAT exposure most probably suggest activation of drought-related signalling prior to measurable physiological impairment. Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defence molecule and a signalling molecule. Review of the literature indicates that a stressful environment results in an overproduction of proline in plants, which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance; stabilizing membranes, thereby preventing electrolyte leakage; and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus

preventing oxidative burst in plants. Reports indicate enhanced stress tolerance when proline is supplied exogenously at low concentrations (Hayat et al., 2012). The pattern observed in our experiment indicates that PBAT MPs may induce an early molecular-level stress response preceding overt drought symptoms. It is also possible that PBAT degradation products may directly influence proline synthesis, rather than through soil-induced drought; however, this requires further investigation.

Proline accumulation under water deficit is primarily mediated through abscisic acid (ABA)-dependent signalling. ABA activates drought-responsive transcription factors such as *DREB2A*, *AREB/ABF*, and *NAC*, which upregulate *P5CS1* (Δ^1 -pyrroline-5-carboxylate synthetase 1)—the key enzyme catalyzing proline biosynthesis from glutamate—while concurrently downregulating *ProDH1* (proline dehydrogenase 1), which limits proline degradation (Bandurska & Stroiński, 2003; Muhammad Aslam et al., 2022). The resulting proline accumulation enhances osmotic adjustment, stabilizes cellular proteins and membranes, and mitigates reactive oxygen species (ROS) damage. Importantly, this response typically precedes more visible symptoms such as leaf wilting or reduced photosynthetic efficiency, underscoring its sensitivity as an early indicator of stress activation.

Emerging evidence suggests that MP exposure can influence ABA-related pathways. In maize, integrated metabolomic and transcriptomic analyses under both conventional and biodegradable MPs showed enrichment of plant-hormone signal transduction and altered ABA metabolism (Sun et al., 2025). Similarly, in lettuce grown in soil mesocosms, PBAT MPs induced stress responses even at 0.025 % (w/w), reflected by reduced chlorophyll content, increased superoxide dismutase (SOD) activity, and elevated malondialdehyde (MDA) levels (Adamczyk et al., 2024). Collectively, these studies support the hypothesis that biodegradable MPs may perturb hormone-regulated drought signaling.

Future research should focus on molecular biomarkers of drought stress to validate this mechanism in sunflower. Specifically, expression profiling of genes involved in ABA biosynthesis and perception (*NCED*, *PYR/PYL/RCAR*), transcriptional regulation (*DREB2A*, *RD29A*, *LEA*), and osmoprotectant metabolism (*P5CS1*, *ProDH1*)—alongside targeted hormone quantification (ABA, jasmonic acid, salicylic acid)—would provide a comprehensive understanding of whether PBAT-based MPs activate conserved ABA–proline drought-response networks in sunflowers (Yue et al., 2025). Also, reactive oxygen species, such as hydrogen peroxide could be measured as a biomarker of long-term drought (Huseynova et al., 2015).

4.4. Effects on soil physicochemical properties and enzyme activity

It has previously been shown that MPs might alter soil properties such as soil pH, water-holding aggregates, bulk density, hydraulic conductivity, particle cohesion and pore structure, which impact the soil's water supply and WHC, as well as enzyme activity (de Souza Machado et al., 2018; Qiu et al., 2022; Wang et al., 2023; Yi et al., 2021). We observed an increase of WHC at 1 % of all three MPs (on average by 5–6 %). The increase of WHC is consistent with previous studies that reported an increase of WHC at 2 % (de Souza Machado et al., 2018), and at 5 % PE from mulching films and PP from frost cover (Jemec Kokalj et al., 2024; Šmídová et al., 2024). Increased WHC indicates a lower water availability for plants in the MP-spiked soil (van Loon et al., 2025) which goes in line with the observed indications for drought-related stress (higher proline accumulation) for PBAT-based MPs.

Our results also showed an increase in pH after 8 weeks of exposure to PBAT_IND at 0.5 % and 1 % w/w, and a trend of increase at PBAT_HOME. This change in pH could be attributed to the chemical composition of starch-based PBAT, which may undergo hydrolysis and microbial degradation, releasing alkaline byproducts (Gharahi & Zamani-Ahmadmohammadi, 2022; van Kleunen et al., 2020). While also in previous studies an increase in soil pH was observed at PBAT concentrations above 0.5 % w/w (Dissanayake et al., 2024; Li et al., 2022b), some studies also indicate a decrease in pH at higher PBAT concentrations (Boots et al., 2019; Jemec Kokalj et al., 2024; Šmídová et al., 2024). Although significant differences in pH were observed for PBAT MPs, it should be noted that this does not necessarily affect the growth and development of sunflowers, which grow best in soil with a neutral to slightly acidic pH (Sutradhar et al., 2014). Minor variations in pH levels, however, could influence the composition of soil microbial communities, potentially creating a modified environment for plants. Further research is required to determine whether these pH fluctuations might affect microbial composition in our soil, plant growth, and development.

It is also important to investigate whether soil pH influences respiration-related enzymes, such as dehydrogenase (DH) and urease (UR), which are considered sensitive indicators of microbial activity and soil biochemical functioning. DH reflects the overall oxidative metabolism of viable microbes (Tan et al., 2017), whereas UR provides insights into nitrogen cycling processes (Cordero et al., 2019). Dehydrogenase responses in our study were clearly non-monotonic. PE induced a biphasic pattern, with stimulation at low concentrations (0.05–0.5 %) and inhibition at 1 %. Such biphasic responses have been reported previously for soil enzymes exposed to MPs and are often

interpreted as initial stimulation of microbial metabolism due to improved aeration or altered soil structure, followed by metabolic suppression when MP concentrations disrupt microhabitats, water distribution, or oxygen diffusion. In contrast, PBAT_IND caused a consistent decrease in DH activity at all concentrations, suggesting a more direct or sustained inhibitory effect on microbial oxidative metabolism. This may reflect (i) the chemical nature of PBAT, which can release low-molecular-weight degradation products that microbes must detoxify (Dissanayake et al., 2024), (ii) starch availability, which might initially shift microbial community composition towards amylolytic taxa at the expense of taxa contributing strongly to DH activity (Liang et al., 2021), or (iii) subtle changes in soil pH that may suppress DH in sensitive microbial groups (Wolińska et al., 2012).

The increase in urease activity observed for PBAT_IND at 0.05 % and 1 % may be mechanistically linked to nutrient inputs arising from PBAT–starch degradation. Starch-derived substrates can stimulate microbial growth and upregulate N-cycling enzymes, including urease (Liang et al., 2021), especially under mildly increased pH conditions. The observed pH shift in PBAT_IND-treated soils (up to 7.4) could further enhance urease expression, as urease activity typically increases with increasing pH within the neutral-to-alkaline range (Klose and Tabatabai, 2000). Together, these patterns may suggest that PE primarily alters soil physical structure at higher concentrations, whereas PBAT_IND more strongly and consistently interacts with microbial nutrient metabolism, producing divergent responses in DH and urease.

Although some studies suggest that different types of MPs (e.g. PE vs. biodegradable PBAT) differentially affect soil properties and microbial interactions (Liang et al., 2021), our findings did not show noteworthy differences between PE and PBAT-based materials. We would also suggest further studies to investigate the link between soil enzymes and nutrient cycling in soil, not only plants.

4.5. Integrated response patterns

We finally integrated all data, plant parameters as well as soil properties, using two-way hierarchical clustering based on Euclidean distances. This analysis revealed distinct patterns in the effects of MPs on plant health and stress responses (Fig. 7). Notably, PBAT_HOME and PBAT_IND treatments at 0.5 % and 1 % were clustered together while lower concentrations of all three MP types, as well as all PE treatments showed similar responses to the control group, suggesting that their impact on plant physiology may be minimal. The heatmap clearly shows that PE exhibits very different effects compared to both PBAT-based materials, which show strong similarities in their impacts. The question remains whether this is related to differences in physico-chemical properties of tested MPs. The main difference between tested MPs is the polymer composition, starch content (which is not present in PE) and size. A meta-analysis of 37 studies (Shi et al., 2024) found that smaller MP particles have stronger inhibitory effects on plants, particularly on root activity, photosynthesis, and overall growth, whereas larger MPs mainly damage roots. The authors attribute this to smaller particles having higher absorption rates, larger specific surface areas, and stronger interactions with plants. In contrast, larger MPs may act as physical barriers in soil, altering soil structure and root development, but they are poorly absorbed and transported, thus having minimal impact on chlorophyll content in aboveground tissues. Similarly, Fang et al. (2024) reported size-dependent effects on soil aggregates, with smaller particles exerting greater influence. Our findings, however, contradict these reports: PE particles were smaller than PBAT-based MPs yet caused fewer effects, and two PBAT-MPs of different sizes produced similar outcomes. This suggests that particle size was not the primary driver of effects; instead, chemical composition likely played a role. For instance, starch in PBAT-related MPs may alter microbial communities and soil properties, influencing plant metabolism—a hypothesis that warrants further investigation. The hierarchical clustering analysis highlights the complex interactions between MPs, soil conditions, and

plant responses, emphasizing the need for a holistic approach in assessing their ecological impact (Lozano & Rillig, 2022). Overall, this study confirms that MPs are multifaceted pollutants whose effects likely result from combined factors rather than size alone.

5. Conclusion and outlook

This study demonstrates that high concentrations of PBAT-based biodegradable microplastics can induce stress responses in plants, whereas conventional PE microplastics and lower PBAT concentrations had little effect on sunflower growth and physiology. The concurrent increase in proline levels and altered soil water retention under high PBAT concentrations suggest activation of drought-related signalling pathways, but this merits confirmation with additional molecular biomarkers. To fully elucidate the mechanisms involved, future studies should combine physiological and biochemical assessments with molecular drought biomarkers, including genes involved in ABA synthesis, perception, and osmoprotectant metabolism. Long-term exposure experiments are also needed to evaluate whether biodegradable plastic residues persist and accumulate sufficiently to affect soil–plant interactions at the ecosystem scale. Beyond direct plant effects, microplastics may alter soil physicochemical properties, nutrient cycling, and microbial community composition. Future research should therefore adopt an integrative, cross-disciplinary approach linking plant physiology, soil chemistry, and microbial ecology. Overall, the environmental evaluation of biodegradable plastics must extend beyond their degradability to include their long-term ecological and agronomic consequences, particularly under the combined pressures of microplastic pollution and climate change.

CRediT authorship contribution statement

Teja Pelko: Writing – review & editing, Writing – original draft, Visualization, Methodology. **Jure Mravlje:** Writing – review & editing, Methodology. **Gabriela Kalčíková:** Writing – review & editing, Writing – original draft, Funding acquisition. **Tina Skalar:** Writing – review & editing, Methodology. **Andrej Kržan:** Writing – review & editing, Methodology. **Marjana Regvar:** Writing – review & editing. **Mark Starin:** Writing – review & editing, Visualization, Methodology. **Anita Jemec Kokalj:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Katarina Vogel-Mikuš:** Writing – review & editing, Funding acquisition.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the author(s) used ChatGPT in order to check the English grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.127618>.

Data availability

Data will be made available on request.

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